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ARTICLE

Studying genetic diversity of whitefly *B. tabaci* Egyptian isolates in relation to some worldwide isolates

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Abstract *Bemisia tabaci* (Gennadius) (Hemiptera, Aleyrodidae) is considered to be one of the most damaging pests in agriculture, causing severe losses in crops worldwide, affecting the tropical and sub-tropical regions. Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) was used to assess the genetic diversity between different isolates collected from different regions in Egypt compared with some other worldwide isolates of this insect pest. Out of 12 primers 8 primers from Operon technology have shown to differentiate between 13 collected *B. tabaci* samples from all over Egypt and some other samples collected from different countries with two other populations representing biotypes A and B collected from the US used for biotype demarcation. Using 13 insect samples, RAPD analysis has produced a total number of 72 markers; about 68 polymorphic markers were revealed. The total number of bands obtained for each primer ranged from 4 to 14 within an average of 9 bands per primer. Of the pair wise combination among fifteen populations Ismailia population showed the highest similarity index (0.947), while US biotype A scored the lowest similarity index (0.326). Two major clusters were formed from the UPGMA dendrogram, which was constructed based on Dice similarity coefficient. RAPD-PCR screening demarcated the whitefly population based on the host species and genetic biotypes. Two major clusters have been revealed as A and B with two other minor clusters A1, A2, and B1, B2. Most of the samples collected from Egypt were clustered together in a minor cluster named A1. A1 group is divided into two sub-groups. A1a comprises the populations from Beni-Sweif in Upper Egypt, Ismailia, Kalyobia, El-Fayoum, Tanta, Kafr El-Sheikh, Alexandria, and A1b comprises Spain and Sudan. Group A1a is clustered together based on their host which belongs to the Cucurbitaceae family while Alexandria was separated individually based on its host which is cauliflower. Through the similarity matrix it could be concluded that the populations of Beni-Sweif, Ismailia, Kalyobia, El-Fayoum, Tanta, Kafr El-Sheikh had 80–90% similarity, while the Banha isolate had 30–40% similarity.

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1. Introduction

Whiteflies are insects belonging to the family *Aleyrodidae*. They occur in tropical and subtropical regions where they are pests of more than 900 hosts of horticultural and ornamental crops as well as herbaceous plants [15]. In temperate climates, they are usually pests of protected crops. About 1300 whitefly species in over 120 genera have been described [4,13] but relatively few are virus vectors.

The polyphagous sap-sucking with piercing mouthparts *Bemisia tabaci*, order Hemiptera causing many harmful effects on crop plants either directly by sucking the plant sap which causes weak plant growth and leaf chlorosis as well as wilting, or the sooty molds caused by accumulating the sugar solution produced during the feeding process of whitefly causing black spots which affect the photosynthesis and reduce the plant yield. Or the indirect damage caused by transmitting plant viruses. One-hundred and fourteen virus species are transmitted by limited genera of whiteflies. In the *genus Bemisia*, only *B. tabaci* (Genn.) is a virus vector whereas in the *Trialeurodes genus, Trialeurodes vaporariorum, Trialeurodes abutilonea* and *Trialeurodes ricini* transmit viruses. *B. tabaci* transmits 111 virus species while *T. vaporariorum* and *T. abutilonea* transmit three species each. *B. tabaci* and *T. vaporariorum* are present in the European–Mediterranean region, though the former is restricted in its distribution to the Southern parts of Europe up to the South of France. Of the whitefly transmitted virus species, 90% are begomoviruses, 6% criniviruses and the remaining 4% are in the genera *Closterovirus, Ipomovirus* or *Carlavirus* [10].

Virus transmission by *B. tabaci* is responsible for yield reduction reaching 68% in tomato as has been observed by Aboul-Ata et al. [3], five percent of viruliferous whiteflies led to 46% TYLCV infection. The same percentage of whiteflies led to 68% TYLCV infection tomato field crop in Egypt. In Pakistan Cotton leaf curl disease, from 1992 to 1995 the accumulated losses in this crop in Pakistan were calculated as exceeding $5 billion [5], where cotton covers about 60% of the country's exports, with serious effects on yield and on the nation's economy. Several agricultural practices such as monoculturing and the huge pesticide usage causing reducing agricultural enemies has developed several biotypes that differentially exhibit resistance to pesticide and virulence which was observed to happen worldwide at the same time [6]. Identifying and differentiating these biotypes is a very difficult task morphologically. So molecular markers have been widely developed to identify and compare several populations from different biotypes and different locations. RAPD markers are one of the most cheap and relatively simple and rapid techniques to be used in taxonomic purposes [17]. Also RAPD markers have been identified as an efficient tool to differentiate genetically and geographically isolated population and is mostly useful to study the genetic structure of a population because they capture polymorphisms located in introns which are non coding region [9].

2. Materials and methods

2.1. *B. tabaci* population sampling

Different *B. tabaci* isolates were collected from the northern part of Egypt from 6 governorates from squash crops from Banha, El-Fayoum, Kalyobia, and Tanta. *B. tabaci* were collected from Ismailia feeding on cucumber, from Kafr El-Sheikh feeding on cotton, and from Alexandria feeding on cauliflower, while from the Upper Egypt region were collected from the Beni-Sweif governorate also feeding on squash crop. A total of 15 samples were used for the analysis while the rest of the samples were collected from Iran, Spain, Sudan, and Morocco, and from Braunschweig in Germany were collected from the Poinsettia crop (Table 1). The insects were collected by a hand held aspirator and preserved in extraction buffer in −20 °C until DNA extraction was done.

2.2. DNA extraction

Total DNA was isolated from adult whiteflies for each sample using the high pure PCR template preparation kit (Roche, Mannheim, Germany). Extractions were carried out essentially following the manufacturer's instructions with modifications according to [1].

3–5 whitefly individuals were transferred into a sterile 1.5 ml eppendorf tube and homogenized with a sterile micro pestle in 15 µl tissue lysis buffer. After addition of another 35 µl lysis buffer and 10 µl proteinase K (20 mg/ml), the whitefly homogenates were gently mixed and incubated for 1 h at 55 °C and the extraction process was completed as described earlier by Abdullahi [1]. Aliquots of the DNA preparations were analyzed by agarose gel electrophoresis to assess the integrity and the quantity of insect genomic DNA.

2.3. RAPD-PCR

To identify and to determine the phylogenetic relationship between different isolates of whiteflies, twelve random 10-mer primers, Operon A3, A5, A8, A10, B4, B10, B11, B20, C5, C10, H16 (Table 2). 9 were used for RAPD PCR analysis resulting in a reproducible banding pattern. RAPD analyses were carried out according to [1]. Amplification reactions were carried out in 50 µl reaction mix, containing a final concentration of 1.25 mM dNTPs, 25 mM $MgCl_2$, Taq polymerase 3U/µl, 5 µl of 10× Taq polymerase buffers, 1 µl of 10 µM Primer, and DNA of 5 µl then the reaction was completed up to 50 µl. RAPD analysis was performed using 10-mer primers purchased from Operon Technologies Inc. California, USA. Amplification was performed using a thermocycler (Biorad,

Table 1 Sampling locations of whitefly population in relation to their host plants.

Species/biotype	Location	Host
Bemisia tabaci	Banha	Squash
Bemisia tabaci	Braunschweig	Poinsettia
Bemisia tabaci	Beni-Sweif	Squash
Bemisia tabaci	El-Fayoum	Squash
Bemisia tabaci	Ismailia	Cucumber
Bemisia tabaci	Kalyobia	Squash
Bemisia tabaci	Alexandria	Cauliflower
Bemisia tabaci	Tanta	Squash
Bemisia tabaci	Kafr El-Sheikh	Squash
Biotype B	US	Squash
Biotype A	US	Squash

Table 2 Total number of markers and percent of polymorphism developed among *B. tabaci* populations from different regions worldwide by RAPD primers.

Primer name	Total markers generated	Polymorphic markers	Monomorphic markers	Polymorphism percentage
Operon A3	12	11	1	91.6
Operon A5	4	3	1	75
Operon B10	5	5	0	100
Operon B20	14	13	1	92.8
Operon B11	10	10	0	100
Operon C10	7	7	0	100
Operon C5	9	8	1	88.8
Operon H16	11	11	0	100
Total markers	72	68	4	94.4

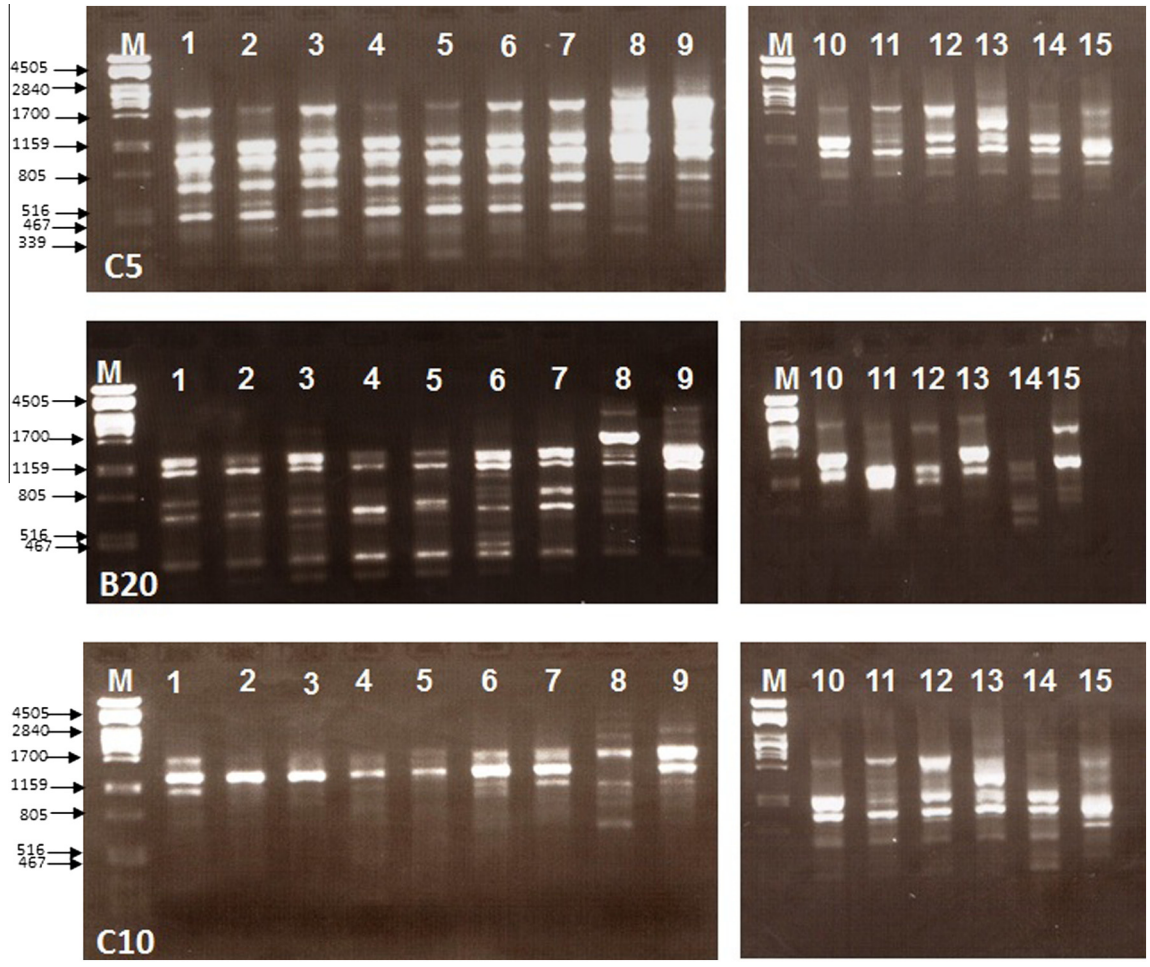

Figure 1 A representative RAPD gel set for Egyptian whitefly populations under investigation M: λHindIII marker, 1–13 samples as follows; (1) Beni-Sweif, (2) El-Fayoum, (3) Ismailia, (4) Kalyobia, (5) Alexandria, (6) Tanta, (7) Kafr El-Sheikh, (8) Spain, (9) Sudan, (10) Morocco, (11) Iran, (12) Banha, (13) Braunschweig, (14) US-B, (15) US-A. Primers used are written on every gel (1) Operon C5, (2) Operon B20, (3) Operon C10.

USA) programed as follows: one initial cycle for denaturation on 94 °C for 2 min, 40 cycles each of: 92 °C for 20 s followed by annealing of 38 °C for 15 s, Ramp 0.3 °C/s to 72 °C for 1 min, and an extension cycle for 7 min on 72 °C. PCR produced were separated on 2% agarose gel electrophoresis.

2.4. Analysis of amplification products and data scoring

RAPD profiles were scored individually for the 8 operon primers and were subjected for cluster analysis and a phylogeny was derived by calculating the relationships between the banding

patterns using the SPSS program. The scoring was done as presence (1) and (0) absence. The matrix was used to calculate the dice similarity coefficient. Clustering was done using the Unweighted Pair-Group Method with Arithmetic Averages (UPGMA). The dendrograms below demonstrate the phylogenetic relationship between different whitefly insect samples collected from Egypt.

3. Results

Eight out of twelve primers screened produced clear bands through RAPD amplification, produced gels were used for analysis using the SPSS program. These nine primers Table 2 have produced a total of 72 markers. The total number of clear bands obtained from each primer ranged from 4 (Operon A5) to 14 (Operon B20) with an average of 9 bands per primer (Table 2). The size of the amplicons ranged from 2900 bp to 350 bp and the clearest bands were around 850 bp. Amplification patterns are shown in Figs. 1 and 2. Genetic relationships between populations are shown in Table 3 representing the dendrogram based on the Dice similarity coefficient.

The similarity coefficient based on 72 RAPD markers ranged from 0.326 to 0.947. Among the thirteen sample populations through the pair wise combination between them and the Ismailia population showed the highest similarity index (0.947), while US biotype A scored the lowest similarity index (0.326), see Table 3. An UPGMA dendrogram based on Dice similarity coefficient was constructed to study the phylogenetic relationship between the thirteen samples. The dendrogram in Fig. 3, revealed two major clusters A and B. PCR analysis provides a population demarcation based on different biotypes and geographical distribution. The major cluster A is divided into two minor groups A1 and A2. A1 group is divided into two sub-groups. A1a comprises populations from the Beni-Sweif in Upper Egypt, Ismailia, Kalyobia, El-Fayoum, Tanta, Kafr El-Sheikh, and Alexandria. A1b comprises Spain and Sudan; these populations are considered to be closely related to *B. tabaci* biotype B from US which represents the minor group A2. The Group of cluster B comprises two minor groups B1 and B2. B1 comprises populations from Banha, Iran, Morocco, and Braunschweig and B2 contains biotype A which was used for demarcation. The dendrogram revealed that all populations collected from northern Egypt cluster together despite differences in the host as they are all collected from the Cucurbitaceae family such as squash and cucumber. The population from Alexandria was collected

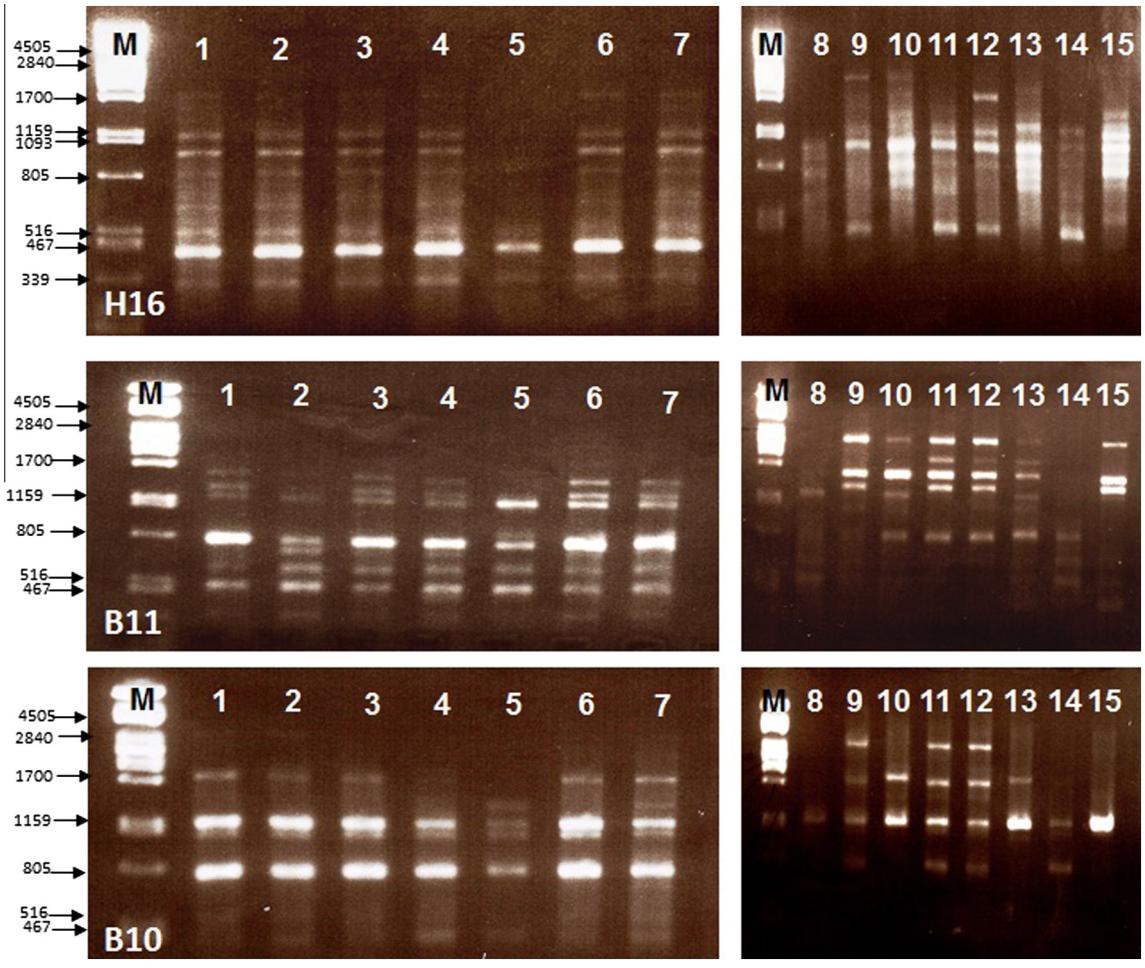

Figure 2 A representative RAPD gel set for Egyptian whitefly populations under investigation M: λHindIII marker, 1–13 samples as follows; (1) Beni-Sweif, (2) El-Fayoum, (3) Ismailia, (4) Kalyobia, (5) Alexandria, (6) Tanta, (7) Kafr El-Sheikh, (8) Spain, (9) Sudan, (10) Morocco, (11) Iran, (12) Banha, (13) Braunschweig, (14) US-B, (15) US-A. Primers used are written on every gel (1) Operon H16, (2) Operon B11, (3) Operon B10.

Table 3 Dendrogram based on Dice similarity coefficient showing the relationship among *B. tabaci* population collected from different regions in Egypt with other collected worldwide populations.

Beni-Sweif	1														
El-Fayoum	0.921	1													
Ismailia	0.947	0.892	1												
Kalyobia	0.935	0.907	0.907	1											
Alexandria	0.789	0.812	0.754	0.8	1										
Tanta	0.911	0.857	0.857	0.872	0.806	1									
Kafr El-Sheikh	0.923	0.868	0.895	0.883	0.789	0.937	1								
Banha	0.571	0.459	0.557	0.548	0.393	0.594	0.571	1							
Spain	0.667	0.658	0.603	0.676	0.676	0.658	0.64	0.433	1						
Sudan	0.684	0.622	0.622	0.693	0.667	0.701	0.711	0.492	0.685	1					
Morocco	0.559	0.485	0.545	0.537	0.393	0.522	0.529	0.679	0.554	0.485	1				
Iran	0.686	0.588	0.676	0.638	0.508	0.648	0.629	0.764	0.478	0.559	0.733	1			
Braunschweig	0.5	0.455	0.455	0.478	0.393	0.464	0.441	0.679	0.492	0.455	0.724	0.733	1		
US B	0.59	0.61	0.542	0.567	0.556	0.645	0.59	0.565	0.483	0.508	0.431	0.566	0.431	1	
US A	0.533	0.448	0.552	0.542	0.377	0.492	0.5	0.667	0.421	0.448	0.64	0.654	0.56	0.326	1

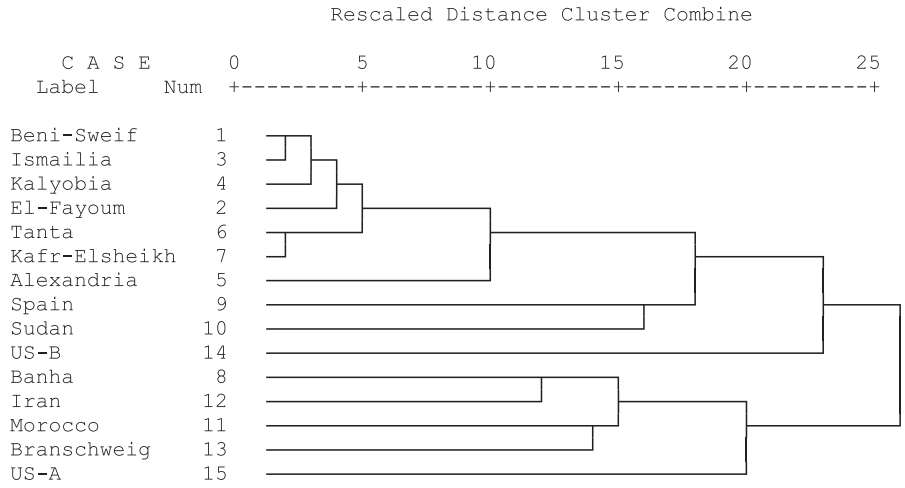

Figure 3 Tree showing Phylogenetic relationship between *B. tabaci* populations in the study.

from cauliflower and the Kafr El-Sheikh population was collected from squash. Genomic DNA RAPD-PCR band patterns also revealed differences between these biotypes using the H16 primer. The B biotype has three bright DNA bands between 250 and 600 bp using the primer Operon C5. A set of unique bands have been generated using several primers recorded as follows; a unique band of 2100 bp by the primer C5, two unique bands by the primer C10 of 2700 bp and 700 bp for the Spain isolate, a unique band of 1700 by the primer A3, 2900 bp, 2400 bp 1950 bp by the primer B20 for the Sudan isolate, 2600 bp band by the primer C10, and a 850 bp for biotype A generated by the primer A3, a unique band characterizing both Iran and Braunschweig isolates of 1700 bp was generated by the primer B10. A band of 650 bp was generated by the primer B20, and a unique band of 900 bp by the primer B11 for the Alexandria isolate. A unique band of 500 bp was generated by the primer B20 of the Ismailia isolate and 400 bp was generated by the primer B20 for the Tanata isolate. A unique band of 700 bp was generated by primer B11 of the El-Fayoum isolate. A unique band of 1600 bp was generated for the Braunschweig isolate. H16 primers and B10 have generated no unique bands in the study.

4. Discussion

Different whitefly populations collected from different regions in Egypt were evaluated for their genetic diversity using RAPD markers. Molecular markers have become very useful for identifying variations between indistinguishable insect populations by means of morphological variations. RAPD technique has been used to identify specific variations between *B. tabaci* variants [11,7,8] and to estimate the closely related populations based on the geographical region and different biotypes [14,2,12]. In this study we are trying to identify the genetic diversity or our Egyptian populations in comparison with different isolates obtained from different regions worldwide from Iran, Morocco, Germany, Spain, and Sudan.

Despite the minor differences in host plants from which our Egyptian populations are collected, the dendrogram showed that all the populations isolated from northern Egypt are clustered together as they are all collected from the Cucurbitaceae family such as squash, cucumber. The population from Alexandria was collected from cauliflower, it was clear that populations collected from squash plants like Beni-Sweif, Kalyobia, Tanta, El-Fayoum and the Kafr El-Sheikh population

and the Ismailia sample from cucumber are clustered together in a smaller minor group which point out to an effect of the type of the crop on the genetic variability of the insect population [16], while the Alexandria population was collected from cauliflower which has been clustered in a separate minor group [16]. Our investigation together with the study of Perumal and Marimuthu [16], which was done in India of different collected populations from different hosts showed that within a narrow geographical region an existing variation could be found and used based on host plants. Our narrow geographical region was represented by isolates collected from the Nile Delta and Alexandria. It has been also observed that nearly all populations collected from Egypt are closely related to *B. tabaci* biotype B including populations collected from Sudan and Spain. While populations collected from Iran and Morocco and from Germany as well as the Egyptian population collected from Banha was closely related to *B. tabaci* Biotype A represented by the sample collected from the US.

5. Conclusion

All insect specimens collected from Egypt and specifically from the Nile Delta governorates were closely related to each other and provide evidence that the *B. tabaci* whiteflies investigated in our study when collected from different regions could be grouped based on their host which is the Cucurbitaceae family members in our case even if a population from upper Egypt also shares the same genetic relatedness based on their host. It is concluded from our study that the most related *B. tabaci* populations to our population are Populations from Sudan and Spain which point to an effect of the geographical distribution and the effect of genetic biotypes on the demarcation of the population, with an emphasis on all collected Egyptian *B. tabaci* which are closely related to biotype B.

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